Copper Anti-inflammatory Drugs in Rheumatoid Arthritis. Part 2.[1] A Potentiometric and Spectroscopic Study of Copper(II) Polyaminodicarboxylate Complexes

Graham E. Jackson * and Mark J. Kelly
Department of Inorganic Chemistry, University of Cape Town, Rondebosch 7700, South Africa

Copper(II) complexes of 3,6,9,12-tetra-azatetradecanedioic acid and 3,6,9-triazaundecanedioic acid have been studied at 25 °C and an ionic strength of 0.15 mol dm^{-3}, using glass electrode potentiometry. A novel formation function has been used as an aid to interpretation of the data. N.m.r. and electronic spectrometry have been used to rationalize the data in terms of solution structures. Computer simulation has been used to predict the copper plasma mobilizing ability of the ligands.

Rheumatoid arthritis is a debilitating disease, afflicting about 5% of the western world's population. There is no cure. Although the disease may be controlled with immunosuppressive drugs or the symptoms treated with anti-inflammatory drugs, more efficient therapeuticals are needed. Sorenson[2] and Jackson *et al.*[3] have shown that copper complexes are effective in reducing the inflammation associated with arthritis.

Recently we have been engaged in computer simulation studies in order to identify those features of a ligand which would make it an ideal vehicle for the delivery of copper to the site of inflammation.[1] The results of this study have led to the conclusion that an ideal ligand should be a linear dicarboxylate, diphenolate, or dialkyl phosphate-substituted polyamine. Herein we report potentiometric and spectroscopic studies of two such ligands, 3,6,9,12-tetra-azatetradecanedioate (ttda) and 3,6,9-triazaundecanedioate (dtda).

Theory

Throughout the analysis of the data we have made extensive use of certain features within the ESTA suite of programs.[4] Some of these features are fairly novel and require some explanation. The deprotonation function $\bar{Q}$ is the average number of protons released per metal ion, as a result of complexation, and is defined according to equation (1), where

$$\bar{Q} = (T_H^* - T_H)T_M \quad (1)$$

T_H and T_M are the total proton and metal concentrations respectively; T_H^*, given by equation (2), is the calculated total

$$T_H^* = [H] - [OH] + \Sigma r\beta_{0qr}[L]^q[H]^r \quad (2)$$

concentration of protons that would be necessary to give rise to the observed pH if no complexation took place. The summation is over all protonated ligand species. In order to evaluate T_H^* it is first necessary to solve for the free-ligand concentration using equation (3). If we define a formation function for the ligand subsystem according to equation (4) then F, the average number of dissociable protons in a complex (assuming that it is the predominant complex), is given by equation 5. Here $\bar{Q}$

$$T_L = [L] + \Sigma q\beta_{0qr}[L]^q[H]^r \quad (3)$$

$$\bar{n} = (T_H^* - [H] + [OH])/T_L \quad (4)$$

$$F = q\bar{n} - \bar{Q}p \quad (5)$$

represents the average number of protons released from the ligand as a result of complexation, while $\bar{n}$ is the average number of protons which would be bound to the ligand in the absence of metal complexation. The difference between the two therefore gives F, the average number of dissociable protons remaining on the ligand after complexation. Clearly, cognizance of metal–ligand stoicheiometries has to be taken.

Two other functions used in this study also need defining. They are $\bar{Z}$, the average number of ligands bound per metal ion, and the plasma mobilizing index p.m.i.[5] given by equations (6) and (7) respectively.

$$\bar{Z} = (T_L - [L])/T_M \quad (6)$$

$$\text{p.m.i.} = \frac{\text{(total concentration of low-molecular-weight metal complex species in the presence of drug)}}{\text{(total concentration of low-molecular-weight metal complex species in normal plasma)}} \quad (7)$$

Results and Discussion

Protonation.—Protonation constants for dtda, ttda, and trien (triethylenetetramine) are given in the Table. The results for trien are in good agreement with those in the literature.[6] With the exception of the last protonation step which occurs beyond the reliable range of our glass electrode, the protonation constants for dtda and ttda show a low standard deviation. The crystallographic R factor indicates the correctness of the protonation model.

For these three ligands the β_{011} is similar, but decreases from trien to dtda (9.88, 9.77, 9.66). It is easy to rationalize these results in terms of the probable protonation scheme given in Figure 1 in which protonation occurs first at a central amine. The decrease in β_{011} can then be attributed to the inductive effect of the acetate groups, being greatest for dtda where two acetate groups are in close proximity to the central amine. This explanation, however, is at variance with the variable pH ^1H (Figure 2) and ^{13}C n.m.r.[7] results. With the addition of the first proton, all resonances are shifted and hence protonation must be occurring to the same degree at all four nitrogen sites. The second step, however, is clearly at $N^1(N^4)$ with the third proton being added to $N^1(N^4)$. The second protonation step of ttda and dtda is significantly weaker than trien, which is consistent with protonation at a terminal amine.

The protonation constants[8] of triethylenetetramine-$N'N''$-

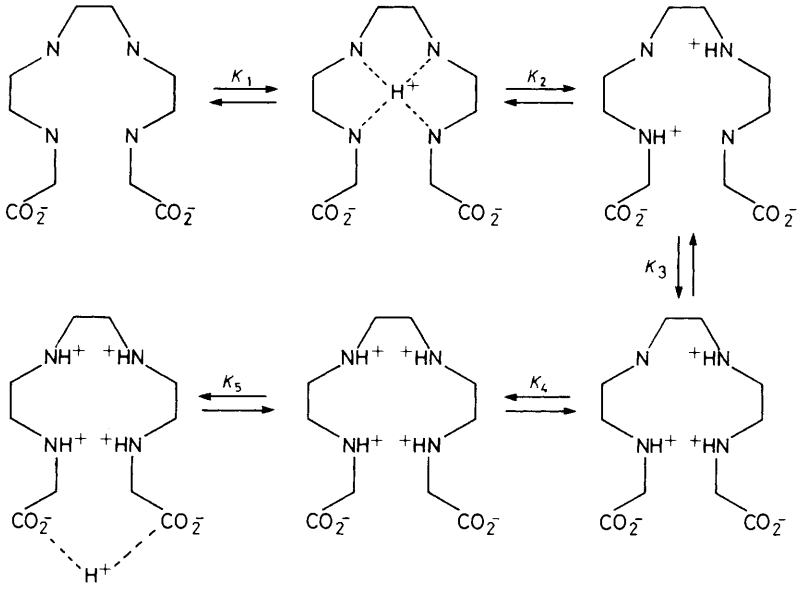

Figure 1. Protonation scheme for ttda

Table. Formation constants determined in this study at 25 °C, I = 150 mmol dm^{-3} NaCl; σ denotes the standard deviation in log β and n is the number of experimental observations used as data for the least-squares calculations. R is the crystallographic R factor. The general formula of a complex is expressed by $Cu_pL_qH_r$

L	p	q	r	log β_{pqr}	σ	n	R
trien	0	1	1	9.880	0.0016	425	0.0017
	0	1	2	19.065	0.0018		
	0	1	3	25.763	0.0025		
	0	1	4	29.207	0.0035		
	1	1	0	20.323	0.0091	406	0.0022
	1	1	1	23.437	0.008		
	1	1	−1	8.61	0.03		
dtda	0	1	1	9.662	0.002	672	0.0028
	0	1	2	18.097	0.005		
	0	1	3	22.349	0.008		
	0	1	4	24.47	0.02		
	1	1	0	19.16	0.01	400	0.0059
	1	1	1	21.35	0.02		
	1	1	−1	8.24	0.02		
dtpa/trien	1	1	0	18.96	0.01	483	0.0026
	1	1	1	21.07	0.02		
ttda	0	1	1	9.766	0.002	800	0.003
	0	1	2	18.603	0.004		
	0	1	3	24.947	0.007		
	0	1	4	28.09	0.01		
	0	1	5	29.53	0.04		
	1	1	0	21.34	0.02	140	0.0024
	1	1	1	24.92	0.01		
	1	1	2	26.67	0.04		
ttda/trien	1	1	0	21.15	0.03	437	0.0038
	1	1	1	24.75	0.02		
	1	1	2	26.81	0.04		
trienda[6]	0	1	1	10.54			
	0	1	2	20.40			
	0	1	3	27.10			
	0	1	4	30.88			
	1	1	0	21.78			
	1	1	1	26.57			

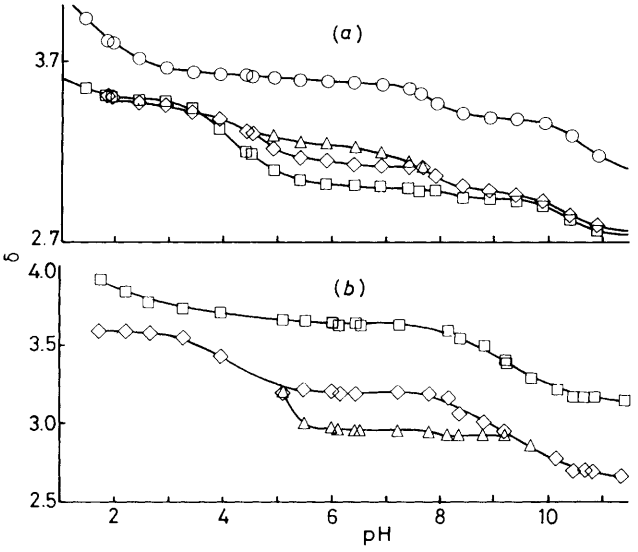

Figure 2. pH Dependence of the ^{1}H n.m.r. chemical shifts for (*a*) ttda and (*b*) dtda. The symbols refer to the protons attached to the following carbons: ttda, C^2, C^{13} (○); C^4, C^{11} (△); C^5, C^{10} (◇); C^7, C^8 (□). dtda, C^2, C^{10} (□); C^4, C^8 (◇); C^5, C^7 (△)

diacetate (trienda) are all significantly higher than our results for the linear analogue and are also higher than those of trien. This is difficult to rationalize on electronic grounds but may be due to entropy changes associated with tertiary (rather than secondary) amines.

Copper.—The formation and deprotonation curves for the Cu–dtda system are shown in Figure 3. At the start of the titration (pH 2), $\bar{Q}$ is >1 indicating that complexation has already commenced. The $\bar{n}$ curve shows that at pH 3.6 there are essentially three protons to be displaced from the ligand while $\bar{Q}$ indicates that at this pH these protons have already been displaced by the metal ion. At higher pH values the $\bar{n}$ and $\bar{Q}$ curves are coincident indicating that no further complexation takes place. The very narrow effective titration range (pH 2—3) is a result of the high stability of the copper complex relative to the pK_a of the ligand. Because of this narrow pH range,

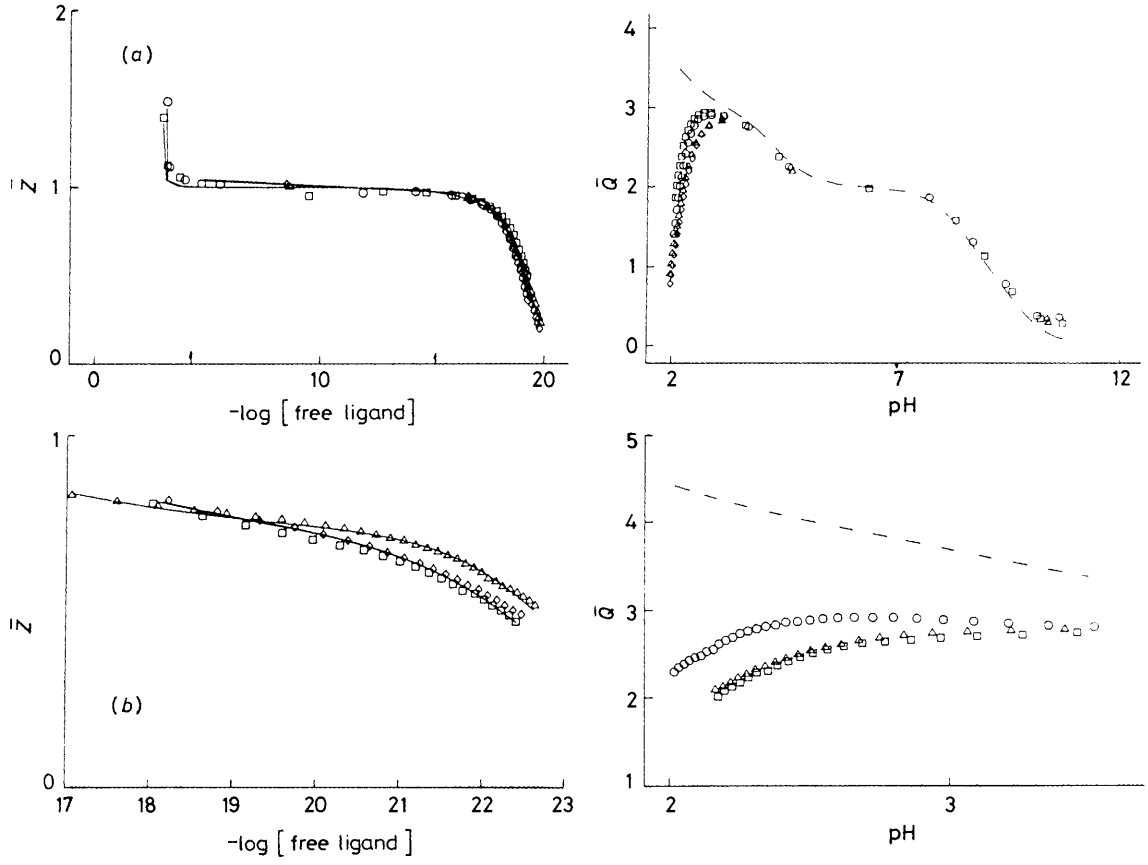

Figure 3. Experimental formation and deprotonation curves obtained at 25 °C and I = 150 mmol dm^{-3} for the systems (*a*) Cu–dtda and (*b*) Cu–ttda. The solid lines represent the theoretical curves calculated using the formation constants given in the Table. The broken lines are $\bar{n}$ curves. For clarity only selected points are plotted: (*a*) [dtda], [Cu] = 3.4, 1.7 (○); 3.0, 1.5 (□); 1.5, 1.5 (△); (*b*) [ttda], [Cu] = 3.0, 1.5 (△); 3.4, 1.7 (○); 1.7, 1.6 (◇); and 2.0, 1.7 mmol dm^{-3} (□)

computer analysis of these data is not ideal. Notwithstanding this limitation, the calculated complex stabilities given in the Table have a reasonably low standard deviation and crystallographic R value.

Because of the limited pH range over which the system was investigated, the study was repeated using trien as a competing ligand.[9] This enabled the titrations to be carried out over a much wider pH range (2—11). The results of this analysis are given in the Table. They are in essential agreement with the binary system and show improved statistics.

Similar results were obtained for the Cu–ttda system, the results of which are shown in Figure 3 and the Table. In this case better results were obtained for the binary system than for the ternary ttda–trien–Cu system.

Comparison of the results for the most predominant ML (metal–ligand) species of trien, ttda, and dtda shows that, as expected, the [Cu(ttda)] complex is the most stable. The increase in stability of the copper complex of ttda over that of trien must be due to the increased denticity of ttda. Comparison with trienda shows that the [Cu(trienda)] complex is only marginally more stable than the [Cu(ttda)] complex even though the basicity of trienda is significantly higher than ttda. Within the complex, ring strain about the tertiary amines may account for this discrepancy. Certainly, it has been shown that in [Ni(edta)]$^{2-}$ (edta = ethylenediaminetetra-acetate) the ligand is quinquedentate, the sixth co-ordination site being occupied by a water molecule.[10]

The potentiometric results show that a significant amount of the protonated M(HL) species is formed at low pH. For this species there is a large difference in stability between trien and ttda [log K = 12.5 and 15.1 respectively for the equilibrium M + HL $\rightleftharpoons$ M(HL)], but little difference between ttda and trienda (log K = 16.03). These results could be accounted for by protonation occurring at a carboxylate site in the case of ttda and trienda. It would also support the hypothesis of ring strain about the tertiary amine of trienda being responsible for the decreased stability of the [Cu(trienda)] complex, as protonation would relieve this strain, making the [Cu(trienda)H]$^{+}$ complex more stable than the [Cu(ttda)H]$^{+}$ complex.

The postulate that protonation of a carboxylate group occurs is also supported by the electronic spectra of the complexes (Figure 4). Over the entire pH range 2—9 there is no change in $\lambda_{max.}$ (624 nm).

For regular octahedral copper(II) complexes[11] the separation of the t_{2g}–e_g orbitals is about 770 for CuO_6 and 550 nm for CuN_6. With tetragonal distortion, as the axial bond lengthens the in-plane ligands move in and the absorption band shifts to shorter wavelength, *e.g.* for [Cu(trien)$(H_2O)_2$]$^{2+}$, $\lambda_{max.}$ = 575 nm.[12] Constraint of the axial bond by being part of a chelate ligand results in a shift to longer wavelength. In our case a $\lambda_{max.}$ of 624 nm is consistent with equatorial co-ordination of the four amine groups and axial co-ordination of the two carboxylate groups.

Above pH 4, where according to the potentiometric results only the ML species is present, there is no change in the electronic spectrum. Below pH 4, however, $\varepsilon_{max.}$ increases and then decreases as the entire complex dissociates. The increase in $\varepsilon_{max.}$ corresponds to the formation of the M(HL) species. There is no change in $\lambda_{max.}$. For [Cu(trien)]$^{2+}$ $\lambda_{max.}$ shifts from 575 to 610 nm upon protonation of a co-ordinated amino group.[13]

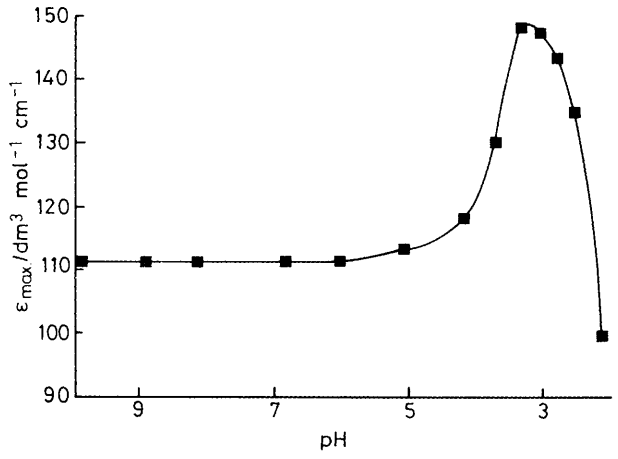

Figure 4. Variation in $\varepsilon_{max.}$ with the pH for the Cu–ttda system. [Cu] = 1.6, [ttda] = 1.6 mmol dm^{-3}

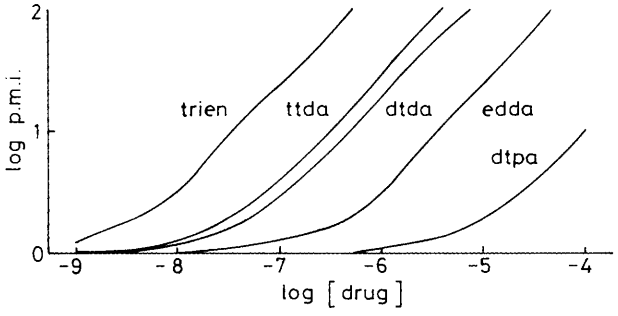

Figure 5. P.m.i. curves for ttda, dtda, and related ligands. edda = ethylenediamine-*NN'*-diacetate

The change in $\varepsilon_{max.}$ and the lack of change in $\lambda_{max.}$ can be explained by protonation occurring at one of the carboxylate sites. Release of one of the axially co-ordinated carboxylate groups from the metal ion increases the tetragonal distortion of the complex resulting in an increase in absorption coefficient. For the ML species $\varepsilon_{max.}$ = 110 while for the M(HL) complex $\varepsilon_{max.}$ = 176 dm^3 mol^{-1} cm^{-1}.

The objective of this research was to produce a ligand which would mobilize copper *in vivo*. Figure 5 shows the computed increase in low-molecular-weight copper caused by ttda, dtda, and related ligands. These calculations were based on an updated model of blood plasma.[5,14,15] From this we see that ttda and dtda should be efficient mobilizers of copper, 1 000 times better than diethylenetriamine-*NNN'N''N''*-penta-acetate (dtpa). Surprisingly, even though ttda forms more stable copper complexes than trien, it is less efficient at mobilizing copper in our blood model. The reason for this is the high concentration of zinc in blood plasma and the relative stability of the copper and zinc ttda complexes. Notwithstanding this we predict that the ttda complex should be stable *in vivo*, and be better at causing a tissue redistribution of copper than trien since the predominant complex, under physiological conditions, is neutral.[3]

Experimental

Solutions for potentiometry were prepared using degassed, double glass-distilled, deionized water. All titrations were carried out under an atmosphere of purified nitrogen. The ionic strength was maintained at 150 mmol dm^{-3} using NaCl. Analytical grade reagents were used throughout the potentiometric titrations. Copper(II) chloride solutions were prepared as required and standardized against edta, sodium hydroxide solutions (100 mmol dm^{-3}) were prepared weekly from standard ampoules under nitrogen and standardized using potassium hydrogenphthalate. Similarly, stock solutions of HCl were prepared and standardized against NaOH and borax.

Potentiometric titrations were carried out in a double-walled vessel, thermostatted at 25 °C. Measurements were made on a Radiometer PHM84 research pH meter equipped with a Metrohm glass electrode and a calomel reference electrode with a renewable liquid junction of 150 mmol dm^{-3} NaCl. Electrode calibration was according to the method of May *et al.*[16]

The compounds ttda and dtda were prepared as the hydrochloride salts[17] and standardized potentiometrically using a Gran plot.[18] Because of the tendency of these ligands to form lactams in acid solution, sufficient ligand to give initial concentrations of between 1.5 and 3.4 mmol dm^{-3}, and the appropriate amount of NaCl, were weighed directly into the titration vessel. Distilled water was then added and the pH immediately adjusted to approximately 11. If copper ions were to be included in the titration, they were added at this stage. Analysis of the data obtained, using the above method, showed that the copper interaction with the ligands was very powerful, with, in the case of ttda, the complex being 50% formed even at pH 2. For this reason the reaction was also studied using trien as a competing ligand. In this case, the same procedure as above was followed, with solid trien (fractionally crystallized as the tetrahydrochloride)[19] also being weighed into the titration vessel.

Data were analysed on a Sperry/Univac 1100 computer using the ESTA suite of programs.[4] MAGEC[16] was used to determine pK_w from strong acid–strong base titrations. A value of −13.76 was obtained and used throughout. N.m.r. spectra were recorded in D_2O solutions on a Varian VXR200 spectrometer using t-butyl alcohol as an internal reference. The pH of the solutions was measured using a microcombination electrode and adjusted using NaOD or DCl. Electronic spectra were recorded on a Philips PU 8700 spectrophotometer under the same conditions as for the potentiometric study.

Acknowledgements

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